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Food-borne Diseases and Techniques to Detect Food-borne Pathogens and Their Limitations

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Abstract

Food safety is a global health concern. Food is one of the major sources of microbial pathogens in the developing regions. Food borne diseases or infections have increased over a year and ultimately resulted into severe health problems. Different type of food borne infections is detected by different type of microbes or pathogens contaminating the food items. Therefore, it is required to detect the pathogens in foods and recognition of problems associated with health and safety.

Hence, variety of techniques has already been developed to detect food borne pathogens or microbes as it is important in analysing the food samples. The detection of food borne pathogens by conventional methods is time consuming, tedious and laborious whereas rapid methods are time efficient, sensitive and much more specific.

In general, these techniques play a vital role in preventing and treating the food borne diseases. The aim of this comprehensive literature is to give an overview in the field of food borne pathogen detection.

1. Introduction

Food-borne diseases or infections are most commonly caused by the consumption of contaminated food & water; this contamination is caused by the pathogens and their toxins which are called food-borne pathogens including bacteria, fungi, virus and parasites
(Zhao *et al*, 2014).

In the US, 31 food-borne pathogens have been identified. It has been estimated that the primary cause of the diseases are viruses whereas the basic cause for hospitalization and deaths are bacteria (Schallan *et al*, 2011).

E. coli 0157:H, Salmonella entrica, Staphylococcus aureus, Listeria monocytogenes, Vibrio spp., Shiga toxin producing E. coli (STEC), Bacillus perfringens, Campylobacter jejuni, Clostridium perfringens are the food-borne pathogens which are commonly responsible for causing disease outbreaks (Law *et al*, 2015).

According to 2015 WHO report on the evaluation of the global load of food borne illnesses 31 food-borne pathogens including bacteria, viruses, parasites and toxins at global and regional level (http://www.who.int/mediacentre/factsheets/fs399/en/).

Reported by WHO (2015), 600 million people (one in ten people in the world) become sick after having contaminated food items and estimated that 4,20,000 die every year. 550 million people fall sick and 2,30,000 die every year because of diarrhoeal diseases (http://www.who.int/mediacentre/factsheets/fs399/en/).

According to food and drug administration (FDA), there is a category of food which should be avoided by the people who are at greater risk of food borne illnesses, example (http://www.fda.gov/Food/FoodborneIllnessContaminants/PeopleAtRisk/ucm352830.htm#FS5):

a) Meat or poultry which is raw or undercooked
b) Refrigerated smoked or partially cooked seafood and raw fish or shellfish (and their juices also).
c) Raw or unpasteurized milk and their products like cheese, yoghurt etc.
d) Foods having raw or undercooked eggs and raw or undercooked eggs.
e) Vegetables not washed.
f) Vegetables or fruit juices which are not pasteurized.

Table: 1. List of certain food-borne microbes involved with outbursts from contaminated food items (Adapted from **Law *et al*, 2015**)

Bacteria	Virus	Parasite
Bacillus cereus	Astrovirus	Cryptosporidium parvum
Campylobacter jejuni	Hepatitis A virus	Entamoeba histolytica
Clostridium botulinum	Hepatitis E virus	Taenia solium
Escherichia coli	Norovirus	Toxoplasma gondii
Listeria monocytogenes	Rotavirus	Trichinella spiralis
Salmonella enterica		
Vibrio cholera		
Vibrio parahaemolyticus		
Vibrio vulnificus		

There is an increasing demand for food safety and sanitation as the food-borne pathogens and food-borne illnesses are posing threat to the human health and life. Food safety and hygiene have become the major issue to be taken into consideration to have healthy and fit life.

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To ensure food safety, proper analysis and testing of food items are required. Advancement and improvement in Biotechnological techniques resulted in the better ways of food testing and analysis methods. Now days it could be observed that ample of companies are involved in developing the more sensitive, specific and rapid techniques rather than traditional methods for the detection of contaminated food items (Mandal *et al*, 2011).

Traditional or conventional methods for the detection of food-borne pathogens

The conventional or traditional methods for microbial detection depend upon the selective media for the enumeration and isolation of viable bacterial cells in the food item. These methods are basically sensitive, inexpensive with both qualitative and quantitative aspects on the number and nature of the microbes to be detected (Doyle, 2001).

There is a series of basic steps involved in the conventional methods for the detection of microbes like pre-enrichment, selective enrichment, selective plating, biochemical screening and serological confirmation. Hence, it could be clearly observed that these methods are more laborious, time-consuming where media preparation, inoculation makes them more tedious (Mandal *et al*, 2011).

Restrictions/ Problems associated with food analysis

Microbiological testing or analysis of food is a challenging task because of certain limitations which pose difficulty in detection, example (Doyle, 2001):

a) In foods, bacterial cells are not evenly distributed.
b) Food matrices with heterogenecity.
   Constituents like proteins, carbohydrates, fats etc
   Foods with different physical state
   Proper mixing is hindered by different viscosity of fats and oils
c) The indigenous microbes which do not pose any health risk but their existence often interfere with the selective identification and isolation of specific pathogens present in low numbers.

These problems could be overcome by concentrating and separating the microbes prior to detection which will help in removing the inhibitory substances present in matrix and will ultimately lead to the time-saving sensitive detection of pathogens. Separation means separating the selected population from the complex mixture whereas concentration involves the sample preparation with reduced sample volume. This process will be resulted into the low volume sample with high recovery of viable bacterial cells (Ruben *et al*, 2001). There are several methods available like Ab-based, physical & chemical based separation and concentration of microorganisms from complex sample matrix; these methods shorten the detection time for pathogens as well as more efficient by concentrating the target microbes (Mandal *et al*, 2011).

Rapid Techniques

For the safety of consumers, the rapid detection of pathogens or microbes in food is very important. Conventional methods for the detection of food borne pathogens depends upon the tedious process of growth of microbes in culture media along with isolation, biochemical identification etc. The latest advancement in technologies leads to isolation, detection and identification which are more convenient, sensitive and specific than conventional assays (at least in theory). These techniques are frequently known as "Rapid Techniques" which involve antibody & DNA-based tests, biochemical kits and tests which are basically the modifications of the conventional methods to hasten the analysis procedures (Peter Fang).

Rapid detection techniques have been categorized into the following (Law *et al*, 2015):

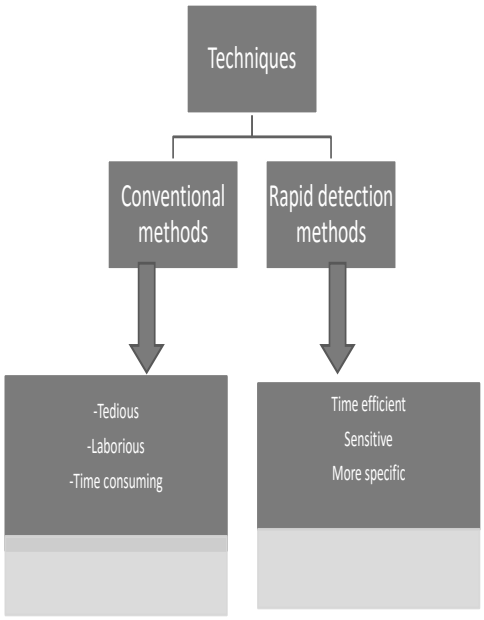

Fig: 1. Techniques

A) Nucleic acid based methods (NABM)
- Polymerase chain reaction (PCR)
- Multiplex PCR
- Real Time quantitative PCR (qPCR)
- Nucleic acid sequence-based amplification (NASBA)
- loop-mediated isothermal amplification(LAMP)
- Microarray technology.

B) Biosensor based methods
- Optical Biosensors
- Electrochemical Biosensors
- Mass based biosensors

C) Immunological based methods
- Enzyme-Linked Immuno sorbent assay(ELISA)
- Lateral flow immunoassay

A) Nucleic acid-based methods (NABM)

For pathogen detection, NABM is more promising in its high specificity, fast results and low detection limits. This technique was developed in the mid-1980s and rapidly achieved widespread application in the field of pathogen detection (Lui *et al*, 2009).

This method basically works by hybridizing the target DNA or RNA (Nucleic acid) sequence in the target pathogen to a synthetic oligonucleotide i.e. primers or

probes which is complementary to the target sequence (Law *et al*, 2015).

A primary limitation in detection of food-borne pathogen/microbe during PCR is the need of an extremely clean DNA template as various contaminants present in food samples can interfere with DNA polymerase activity to hinder the process and generates false negative results (Velusamy *et al*, 2010; Boer & Beumer, 1999).

- **Polymerase chain reaction (PCR)**

It is basically defined as the in-vitro amplification of the nucleic acid and was developed by Mullis and Faloona. The method involves the thermal cycling of repeated heating and cooling of the reaction for the melting of the DNA. Whereas RT-PCR utilizes fluorescent technology using dyes like SYBR Green I or SYBR Gold, fluorescent probes, molecular beacons and scorpion probes. The fluorescent signals generated during this mechanism are directly proportional to the amount of the product produced by the PCR. RT-PCR (Real Time Polymerase chain reaction) is used for both qualitative and quantitative analysis. And this technique is rapid, accurate & highly sensitive (Akbar S Khan, 2014).

- **Multiplex PCR (mPCR)**

It is the method which involves the simultaneous in-vitro amplification of more than one locus in the single reaction for the rapid detection of microbes. In this, primer concentration and designing play a vital role to produce reliable yields of the PCR products. Multiplex PCR is useful in identifying the certain microbial communities (Zhao *et al*, 2014).

- **Nucleic acid sequence-based amplification (NASBA)**

It is basically an isothermal RNA-specific amplification method in single step which amplifies mRNA into double stranded DNA. It has been proved that NASBA successfully detect both bacterial and viral RNA in clinical samples. Combination of standard NASBA technology and molecular beacons generate an RT detection system (Polstra *et al*, 2002).

- **Loop-mediated isothermal amplification (LAMP)**

LAMP is a technique which is a novel and employs the amplification of DNA with more specificity, efficiency and rapidity in the presence of isothermal conditions. This process involves the use of DNA polymerase and a specially designed set of four primers which recognizes a total of six distinct sequences on the target DNA. This method is based on auto-cycling displacement DNA synthesis and the final products will be cauliflower like DNA structures with multiple loop DNAs bearing many inverted repeats of the target. The final products can be visualized by agarose gel electrophoresis staining with SYBR Green I (Notomi *et al*, 2000).

- **Oligonucleotide DNA microarray**

This is a technique which is widely used in the detection of food-borne pathogens (Law *et al*, 2015).

Microarray, comprises of a chip which is a few squared centimetre, bearing upto hundreds of thousands of probes (sequences range from 25 to 75 bps). The sample (mRNA, cDNA, PCR products or genomic DNA) which has to be investigated is labelled with a dye (eg. fluorescent or radioactive dye) and denatured to generate single strand fragments which will hybridize to the array by binding to their corresponding DNA probe. The probe and sample complex will produce the fluorescence signals. The fluorescence intensity is proportional to each nucleic acids fragment concentration (Lauri & Mariani, 2009).

B) Immunological-based method

Immunological techniques for the detection of food-borne pathogens and microbial toxins are based on specific binding of an antibody (Ab) to an antigen (Ag). Monoclonal Abs over polyclonal abs are often more useful for specific detection as the serve an indefinite supply of a single Ab. With advent of monoclonal Abs, immunological detection of contamination became more sensitive, reliable and specific (Zhao *et al*, 2014).

Although immunological-based methods are reliable but the specificity of the assay depends upon quality of the antibody used (Bala Swaminatham & Peter Feng, 1994; de Boer E & Beumer RR, 1999). An enrichment stepis required in case of an inadequate sensitivity to increase the bacterial count in the food sample which in turn increases the time to generate the result (Swaminatham & Feng, 1994).

- **ELISA (Enzyme linked immuno-sorbent assay)**

ELISA is one of the most widely used immunological assys for food-borne pathogen detection because of their accuracy and sensitivity for detecting antigens and haptens. ELISA most commonly used in the detection of toxins, has been developed for *Staphylococcal enterotoxins* A, B, C & E. It has been found to have detection level less than 0.5 µg/100g in ground beef (Zhao *et al*, 2014).

- **Lateral flow immunoassay**

Although ELISA, is one of the most common techniques but still needs variety of equipments and trained personnel. Hence, other reliable method can be conducted for rapid and cheap detection at the site of contamination. Many on-site immunological techniques are based on lateral flow immunoassays eg. Dipstick, immuno-chromatography and immuno-filtration have become a centre of attraction in the field of pathogen, mycotoxin and disease detection in food industry and medicine. In lateral flow immunoassays, the sample flows along the solid substrate (capillary action). Sample encounters a coloured reagent such as Ag or Ab labelled with colloidal latex or gold particles, after the sample is applied to the test. Coloured reagent gets mixed with the sample and transits the substrate, giving lines or zones that have been pre-treated with an Ag or Ab. Coloured reagent bound at the test line or zone depending on the analyte present in the sample (Zhao *et al*, 2014).

C) Biosensor-based methods

Biosensor is an analytical device which is typically associated with three components:

- The sensor platform which is functionalized with a bio-probe to give recognition specificity.
- The transduction platform which gives a measure measurable signal in the events of analyte capture.
- The amplifier which is functionalized by amplifying and processing the signal to quantify the analyte capture.

The biosensors convert the specific bio-recognition into measurable signal. This method is cost-effective, less time-consuming, specific, sensitive and do not require pre-enrichment process (Singh *et al*, 2013).

The common advantage for biosensor system is that the pathogens/microbes do not require labelling prior to

detection, and hence, they are able to produce results rapidly. Unlike conventional methods this system do not need sample pre-treatment (Ivnitski *et al*, 1999).

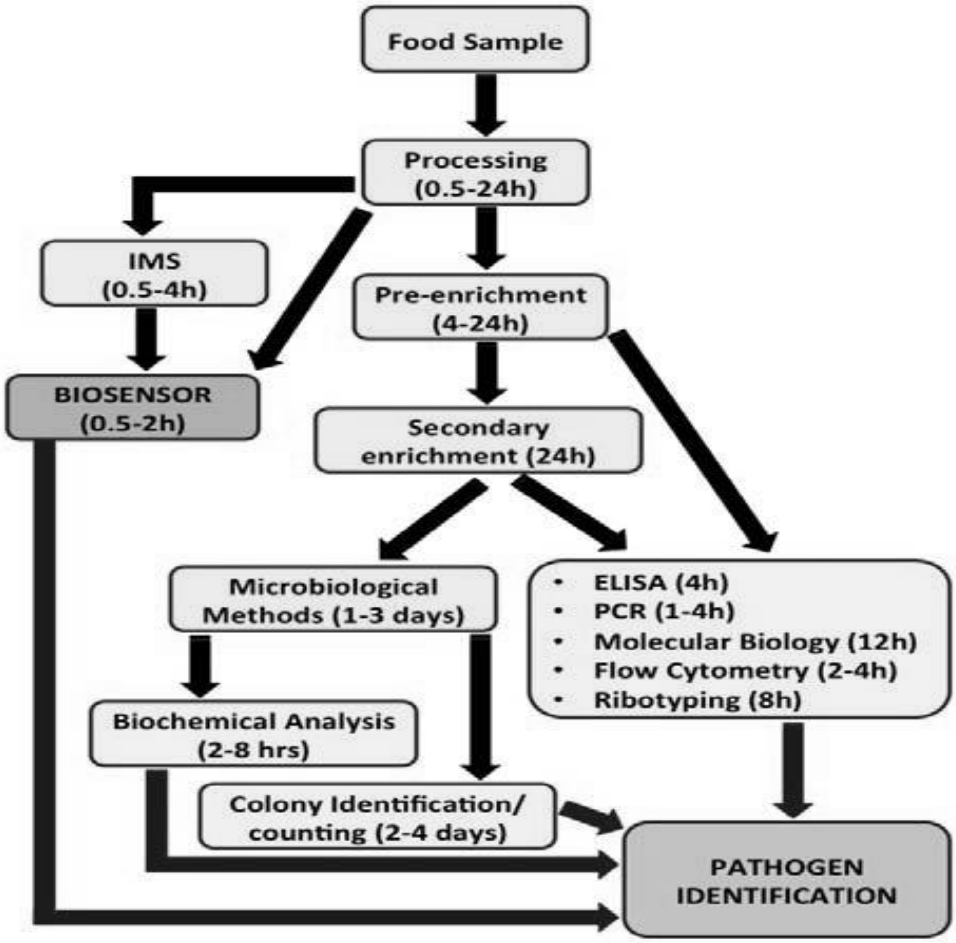

Fig: 2. A fl aken in the detection o erties are modified v f external magnetic field

- **Optical Biosensors**

An optical biosensor is basically a compact analytical device bearing a biological sensing element which is connected to an optical transducer system. This technique is categorized into many subclasses depending on absorption, reflection, refraction, raman, infrared, chemiluminescence, dispersion fluorescence and phosphorescence. In optical biosensors a suitable spectrometer is required to record spectral chemical properties of the analyte. Commonly available method which employs the technique is SPR (surface Plasmon resonance) which uses reflectance spectroscopy for the detection of the food-borne pathogens (Zhao *et al*, 2014).

- **Electrochemical Biosensors**

Transduction based systems, which are used for the identification and quantification of food-borne pathogens (Zhao *et al*, 2014).

Electrochemical transduction techniques can be categorized on the basis of their measured parameter: amperometric (current), potentiometric (potential), impedimetric (impedance) and conductometric. The amperometric sensors are most successful commercially because of their superior sensitivity and better linear range than potentiometric devices (Adley, 2014).

➢ **Amperometric Biosensors**

This electrochemical detection method for food-borne pathogens is most commonly used and offers better sensitivity than other methods. It is made up of two electrodes- reference electrode and working electrode. When voltage is applied then the current produced in the analyte is directly dependent on the rate of electron transfer which shows variation because of ion concentration of analyte. inons in solution are detected by amperometry by measuring the variations in electric current (Singh *et al*, 2013).

➢ **Impedimetric Biosensors**

Electrochemical Impedance Spectroscopy (EIS) biosensors are used to measure the variations in impedance over range of frequencies which are resulted because of bio-molecular interactions. These biosensors are basically used to detect bacteria by monitoring the variations in the surface. Because of the insulating properties of the captured

targets on sensor usually increases the impedance. Unlike amperometry technique, EIS offers label-free detection of microbes but simultaneously it has lower detection limit than the other methods (Singh *et al*, 2013).

➢ **Potentiometric detection**

The bio-recognition process gets converted into a potential signal during potentiometric detection of pathogens. The electrical potential difference or electromotive force (EMF) is measured by the high impedance voltmeter between two electrodes at near zero current. The method employs the detection of extremely small concentration modifications as potentiometry produces a logarithmic concentration response. Although not many potentiometric biosensors were found for the detection of microbes but light-addressable potentiometric sensor (LAPS) has been reported for the detection of pathogens (Velusamy *et al*, 2010).

- **Mass-based biosensors**

Mass-based biosensors allow a piezoelectric crystal that can be induced by an electrical signal to vibrate at a certain frequency. Antibodies coated crystal is used for the antigen of interest. When antibodies coated crystal binds to the antigens from the sample, they decrease its vibrational frequency by a magnitude that corresponds directly to the added mass. A flow-through piezoelectric assay has been observed to detect *E. Coli* with a measuring cycle of 10 minutes; however, the system was not that much sensitive to reliably detect microbe at concentrations less than 106 colony forming units (CFU)/ml (Zhang, 2013).

DETECTION METHODS	ADVANTAGES	DRAWBACKS
A) <u>Nucleic acid-based</u>		
Simple PCR	• High sensitivity • High specificity • Automated • Reliable results	• Affected by PCR inhibitors, Requires DNA purification • Difficult to distinguish Between viable and non-viable cells
Multiplex PCR	• High sensitivity • High specificity • Detection of multiple pathogens • Automated • Reliable results	• Affected by PCR inhibitors • Difficult to distinguish between viable and non-viable cells • Primer design is crucial
Real-time PCR	• High sensitivity • High specificity • Rapid cycling • Reproducible • Does not require post- amplification products processing • Real-time monitoring PCR amplification products	• High cost. • Difficult for multiplex real-time PCR assay • Affected by PCR inhibitors. • Difficult to distinguish between viable and non-viable cells • Requires trained personnel. • Cross contamination may occur
NASBA	• Sensitive • Specific • Low cost • Does not require thermal cycling system • Able to detect viable microorganisms	• Requires viable microorganisms • Difficulties in handling RNA
LAMP	• High sensitive • High specificity • Low cost • Easy to operate • Does not require thermal cycling system	• Primer design is complicated • In sufficient to detect unknown or un-sequenced targets
Oligo-nucleotide DNA microarray	• High sensitivity • High specificity • High throughput • Enables detection of multiple pathogens • Allows detection of specific serotype • Labor-saving	• High cost • Difficult to distinguish between viable and non-viable cells • Requires trained personnel • Requires oligo-nucleotide probes and labeling of target genes

B) Biosensor-based		
Optical biosensors	• High sensitivity • Enables real-time ornear real-time detection • Label-free detection system	• High cost
Electrochemical biosensors	• Can handle large numbers of samples • Automated • Label-free detection	• Low specificity • Not suitable for analyzing samples with low amount of microorganisms • Analysis may interfered by food matrices • Many washing steps
Mass-based biosensors	• Cost effective • Easy to operate • Label-free detection • Real-time detection	• Low specificity • Low sensitivity • Long incubation time of bacteria • Many washing and drying steps • Regeneration of crystal surface may be problematic
C) Immunological-based		
ELISA	• Specific • Can be automated so that it is more time efficient and labor-saving • Allows the detection of bacterial toxins • Can handle large numbers of samples	• Low sensitivity • False negative results • May result in cross-reactivity with closely related antigens • Pre-enrichment is required in order to produce the cell surface antigens • Requires trained personnel • Requires labeling of antibodies or antigens
Lateral flow Immunoassay	• Low cost and Reliable • Easy to operate • Sensitive and Specific • Allow the detection of bacterial toxins	• Requires labeling of antibodies or antigens

2. Conclusion

Traditional methods for the detection of pathogens/microbes in the food sample are found to be time consuming and laborious. Therefore, various rapid techniques have been developed for the detection of food-borne pathogens/microbes in order to overcome the limitations associated with traditional methods of detection. Rapid detection techniques are found to be very important as they are helping in the prevention of various food-borne diseases or illnesses. Rapid detection methods are generally more specific, sensitive, time & labor-saving and reliable than the traditional methods of detection. However, rapid detection methods also have several limitations along with the advantages as summarized in **Table 2**. Hence further advancements on the effect of different combinations of rapid techniques for the detection of food-borne pathogen are required in order to generate the most effective and accurate detection techniques.

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